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Effects of different carbohydrate supplementation on water quality and growth performance of common carp (*Cyprinus carpio*) in biofloc system

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Abstract
A 90- day outdoor trial was conducted in cemented tanks (2.4 m^3) with 10 cm soil base to evaluate the performance of different carbohydrate supplementation on water quality and growth performance of common carp fingerlings under mid-hill altitude. Six carbohydrate sources *viz.* ricepolish (T_1), wheat flour (T_2), sweet potato (T_3), yam (T_4), tapioca (T_5), and rice husk (T_6) were used to maintain the optimum C:N ratio of 10:1 to produce biofloc within the culture system. Common carp fingerlings (9.1±0.2 gm) were stocked @ 10 nos. /m^3 and fed with formulated feed (20% CP) @ 4% body weight daily. During the experiment there was no aeration provided and water also not exchanged throughout the trial period. A control (C) was maintained without addition of carbohydrate source. The study indicated a significant reduction ($p<0.05$) in total ammonia nitrogen (TAN) load in water for the all treatments (T_1 to T_5) compared to control. There was a significant difference in water quality parameters *viz.* pH, dissolved oxygen, free carbon-di-oxide (CO_2), alkalinity, and hardness among the treatment groups. The total dissolved solids (TDS), and conductivity were not differed significantly between the treatments. Further, significantly lowered ($p<0.05$) feed conversion ratio (AFCR) was found for T_3 to T_5 than T_2, T_6 and control. The maximum growth increment in terms of biomass was observed in T_3 @ 32.12% than T_1 @ 21.45% compared to control. The present study indicates that the addition of tuber crops, rice polish as carbohydrate source with low protein diet at a specific ratio can improves water quality and enhances the growth of common carp.

Keywords: Biofloc, C:N ratio, carbohydrate supplements, tuber crops, water quality

Introduction

North eastern hill region (NEH region) of India suffers 54% shortfall in fish production largely due to practicing extensive fish culture and lack of adoption of scientific fish culture. The seasonal water bodies, the non-availability and high cost of feed (40-60% of operational cost) considered as the main cause for poor adoption of intensive fish culture by the farmers in the north eastern states of India. Moreover, the soil acidity, pH 5.5-6.5, high iron and aluminum content in soil, low alkalinity of water (10 to 30 ppm), high rainfall / flash rain, and siltation are some major problems/reasons of low natural productivity in this region. Intensified aquaculture demands more protein rich feed and 80% of supplementary feed usually lose as uneaten feed and faces to environment [13, 14]. Thus, the water quality deteriorates due to high concentration of metabolites and accumulation of nitrogenous waste [8]. The wastewater causes stress to the fish and results several disease outbreak, mortality and loss of farmers [8, 11]. Biofloc technology is a holistic eco-system approach in aquaculture which utilizes the pond heterotrophic microbial community to improve water quality by absorbing the nitrogenous waste material [3, 6] and maximize feed recycling by maintaining a high carbon/nitrogen (C: N) ratio in the production system [5]. Bioflocs are aggregates (flocs) of algae, bacteria, protozoans, and other kinds of particulate organic matter such as feces and uneaten feed [5, 7]. Biofloc technology is well established in developed countries like Israel, USA where land and water scarcity exists [10]. The works have been mainly carried out on Shrimp, Prawn, Tilapia for sustainable intensive aquaculture to reduce water exchange and feed cost by providing cheap bacterial protein achieved by maintaining C: N ratio in pond [12, 13]. By adding carbohydrate source in appropriate ratio at the intensive culture system biofloc can be easily produce and convert waste into protein rich feed. Therefore, with application of BFT technology, the fish and shrimp digestibility, immunity and welfares can be enhanced with maintaining specific C:N ratio with extra carbohydrate addition [1].

The NEH region are the most diversified areas in India and a huge demand for fresh fish exists. Being the land locked area capture fisheries contribute is very less and aquaculture is restricted to carp culture only. The poor socio-economic condition, allows the fish farmers for adoption of composite fish culture with little input. Therefore, the most reasonable method for increasing the fish production in NEH region through intensification with minimum feed and least impairment to the environment seems to be the Biofloc technology (BFT). Biofloc technology may be an alternate production system to meet the huge demand and overcome the major drawbacks for adoption of intensive fish culture. BFT may also help to increase the overall fish productivity in the region as well as improved the Socio-economic status of fish farmers.

Materials and Methods

Experimental design

A 90 days outdoor trial was conducted in cemented tanks (2.4 m^3) with 10 cm soil base during January to March, 2015 at ICAR-Research Complex for NEH Region Barapani H/Q, fish farm. All the tanks were prepared by sun-drying and adding calcium oxide (CaO) at the rate of 200 kg/ ha and manured with cow dung at the rate of 5000 kg/ha for optimization of pH and enhancement of natural productivity. The water depth was maintained 1.0 meter during the culture periods. The experimental tanks were allocated in a completely randomized design (CRD) consisting of six treatments rice polish (T_1), wheat flour (T_2), sweet potato (T_3), yam (T_4), tapioca (T_5), and rice husk (T_6) in triplicate with a control (C). The fish were fed at the rate of 4% biomass twice a day (09:00 a.m. and 4:00 p.m.) with the prepared pelleted feed which contains 20% crude protein. The feed was provided using a feeding tray which hung into the water column at 30-40 cm below the surface. No aeration was provided in the present experiment.

Preparation of feed and carbohydrate source

The sinking pelleted feed were prepared with locally available ingredients with hand pelletizer. The dried feed was stored in polythene bags at low temperature for further use. The proximate composition of the feed and feed ingredients is given in Table 1. The carbohydrate sources sweet potato, yam and tapioca were procured from local (Barnihat) market. They were cut into pieces, dried at 80^0 C using drier, after proper drying they were powdered and stored in polythene bags and kept at low temperature for further use. The other sources like rice polish, rice husk and wheat flour were also purchased from local market and kept at low temperature in polythene bags.

Table 1: Proximate composition of feed ingredients and feed (g/kg dry matter)

Ingredients	Protein (%)	Lipid (%)	Ash (%)	NFE (%)
Mustard oil cake	32.29	12.03	6.65	49.03
Soybean	48.12	1.65	7.50	42.73
Fish meal	61.55	6.12	12.92	19.41
Wheat polish	16.12	2.36	1.70	79.82
Rice polish	8.45	4.16	12.90	74.49
Feed	20.54	2.23	12.85	64.38

Experimental fish, their survival and growth parameters

Two hundred ten healthy fingerlings of common carp with mean body weight and length of (9.1±0.2 gm) g and 8.50 ± 0.60 cm, respectively were collected from ICAR-RC for NEH region fish farm. The fish were acclimatized for 15 days and fed with the experimental feed (20% CP) twice a day at the rate of 4% of the biomass. Subsequently, the fingerlings were randomly distributed in 21 experimental tanks at the rate of 10 numbers per tank maintaining a stocking density at the rate of 40,000 numbers per hector.

The individual weight and total initial biomass were recorded for all the treatment groups. To monitor the growth and adjust feeding schedule, fortnightly samples of 10 fishes from each tank were collected and weighed batch-wise. At the end of the experiment, feeding was stopped before 24 h. All the fishes were harvested after dewatering the tanks. The fish were caught counted and individual weight as well as final biomass was recorded. The survival, daily weight gain (DWG), apparent feed conversion ratio (AFCR), were analyzed by the following formula:

1. Survival (%) = Final number of fish/Initial number of fish x 100.
2. DWG (g/ day) = (final mean weight − initial mean weight)/ culture days.
3. AFCR = Dry feed intake (g) / Wet weight gain in fish (g).

Water parameters

All the water quality parameters were analyzed fortnightly. Transparency was measured by Secchi disc while pH, electrical conductivity and total dissolved solids were measured by a digital probe-based water analyser (Systronics, India). Other water quality parameters, i.e., dissolved oxygen, free carbon-di-oxide, total alkalinity, and hardness were measured by APHA (2005) method [2]. The total ammonical nitrogen (TAN), and orthophosphate were measured utilizing spectrophotometer (Thermo-fisher) as per standard methods (APHA, 2005) [2].

Statistical analysis

The results for each treatment are expressed as mean ± standard error (SE) of triplicate measurements and analyzed with SPSS (version 16). One-way analysis of variance was performed according to ANOVA procedures. Significant differences between the treatment means were determined by Duncan's multiple range tests. f-value < 0.05 was regarded as significant.

Results and discussion

Effect of different carbohydrate sources on water quality parameters

There was significant reduction (p<0.05) in total ammonia nitrogen (TAN) load in biofloc system (Fig 1) than the control (without carbohydrate supplementation). A significant difference was observed among the treatments for major water quality parameters like pH, dissolved oxygen (DO), free carbon-di-oxide (free CO_2), hardness, alkalinity and transparency (Table 2). The conductivity (EC) and total dissolved solids (TDS) were not significant statistically between the treatments. The pH, alkalinity, and hardness were found in higher side in tuber crop based biofloc system (T_3, T_4 and T_5) than the T_1, T_2 and T_6. The transparency was highest in T_6 and lowest in T_1. There was algal bloom observed in control. The floc volume was ranges from 15-20 ml/L during the culture period in BFT system. The current study is in line with the earlier findings, where the addition of carbohydrate reported to reduce the TAN concentration in tilapia [4], shrimp [14]. It has been reported that heterotrophic bacteria absorb TAN and utilize the supplemented carbon for the production of microbial floc within the culture system [3, 15]. The reduced TAN levels, overall, helped to maintain better water quality in carbohydrate supplemented tanks [9, 10].

Effect of different carbohydrate sources on growth parameters

The survival was higher in T_5, T_3, T_4 and lower in control, T_1, T_2 and T_6 (Table 3). The total biomass was higher in T_5, T_4, T_3 and final mean weight was highest in T_5 and lowest in control. The daily weight gain (DWG) was more in T_5 than other treatments. The apparent feed conversion ratio (AFCR) was significantly lower ($p<0.05$) in T_5 and highest in control. The maximum growth increment in terms of biomass were found @ 32.12% (T_3), 21.45% (T_1), 17.51% (T_5), 17.48% (T_4), 5.89% (T_2) and 2.31% (T_6) compared to control (Fig 2). The results were comparable with the tilapia production utilizing biofloc technology where they achieved 100% fish survival. The net fish production was 45% higher in the BFT tanks than in the control tanks. They reported there was no difference in fish growth/production between 35% and 24% CP fed tanks under BFT. This indicates that biofloc can reduce the high protein demand by supplementing microbial feed and enhance fish production [4, 9]. In the present study the results indicated that with 20% CP feed, the common carp production can be increased by 32% with application of biofloc technology. Biofloc improves the digestive enzyme activities in shrimp, freshwater prawn and tilapia, therefore the feed digestibility increased and enhanced feed conversion ration [1, 16]. The present study also found better AFCR and DWG in biofloc system than the control.

Effect of different carbohydrate sources on plankton abundance

The results were differing significantly in all the treatments for plankton abundance. The association of biofloc and plankton showed in Fig 3. In control, T_1, T_2 and T_6 there was algal bloom and abundance of wastewater plankton community like *Scenedesmus*, *Chlamydomonas*, *Anabaena*, etc observed. But in tuber crops based biofloc system (T_3, T_4 and T_5) there was no algal bloom and abundance of good planktonic community like diatom (*Navicula*, *Meloseria*, *Spirulina* etc) green algae like *Chlorella*, *Pediastrum* etc were found. More over the wastewater planktonic community like *Anabaena*, *Nitzschia* and *Euglena* were not found in T_3, T_4 and T_5 treatments.

There was no literature found on the tuber crop based biofloc system and their effects on plankton communities to compare our result. As tuber crops are alkaline food which supplementation in biofloc system may improves the pH, alkalinity and hardness of water and promotes the diatom growth and prevent harmful algal bloom like, anabaena, euglena. Good plankton communities adding extra value in tuber crops based biofloc system which may improve AFCR and thereby good health of fish. Thus, the overall production was more in tuber crop based biofloc system than control.

Table 2: Water quality parameters

Treatment	pH	DO (ppm)	Free CO_2 (ppm)	Hardness (ppm)	Alkalinity (ppm)	EC (µS/cm)	TDS (ppm)	Transparency (cm)
C	7.56±0.12^{b}	8.0±0.58^{ab}	3.0±0.58^{a}	27.0±0.58^{ab}	62.33±1.45^{ab}	173.90±11.68^{a}	79.03±9.13^{a}	9.17±3.00^{a}
T_1	7.30±0.10^{ab}	10.06±1.15^{b}	3.0±0.58^{a}	28.0±1.00^{a}	86.67±8.82^{b}	171.50±8.21^{a}	76.47±1.34^{a}	15.5±3.00^{ab}
T_2	7.40±0.15^{b}	6.00±1.15^{a}	2.67±0.67^{a}	26.00±1.00^{a}	70.0±5.77^{b}	165.43±8.57^{a}	74.47±12.08^{a}	18.2±2.02^{b}
T_3	7.63±0.09^{b}	6.00±0.58^{a}	2.33±0.33^{a}	29.67±1.45^{ab}	76.67±13.33^{b}	158.80±17.29^{a}	78.90±4.48^{a}	15.6±3.07^{ab}
T_4	7.63±0.09^{b}	6.68±1.20^{a}	2.33±0.33^{a}	32.68±0.67^{b}	71.67±4.4^{b}	167.13±9.90^{a}	75.0±6.32^{a}	18.83±0.33^{b}
T_5	7.63±0.14^{b}	6.68±0.88^{a}	3.68±0.68^{a}	29.33±2.96^{ab}	76.67±8.82^{b}	139.63±13.25^{a}	74.13±5.75^{a}	15.17±2.40^{ab}
T_6	7.03±0.09^{a}	8.36±1.16^{ab}	2.6±0.74^{a}	26.33±0.88^{a}	41.0±0.58^{a}	155.17±15.93^{a}	67.56±11.48^{a}	19.17±0.33^{b}

C=Control, T_1 Rice Polish, T_2 =Wheat Flour, T_3= Sweet potato, T_4=Yam, T_5: Tapioca, T_6: Rice husk,
(Values are mean ± standard error, those that are statistically significant are presented as a,b)

Table 3: Growth performance of Common carp (*Cyprinus carpio*)

Treatment	Initial Mean Weight (g)	Final Mean weight (g)	Survival (%)	Initial Biomass (g)	Final Biomass (g)	Daily weight gain (DWG)	Apparent feed conversion ratio (AFCR)
C	8.97±0.06^{a}	23.93±1.44^{a}	66.67±2.41^{a}	215.43±1.44^{a}	387.40±32.20^{a}	0.16±0.015^{a}	2.07±0.19^{b}
T_1	9.15±0.01^{a}	23.41±2.33^{a}	86.11±7.35^{b}	219.07±0.58^{a}	470.51±68.67^{ab}	0.20±0.05^{ab}	1.74±0.24^{ab}
T_2	9.06±0.07^{a}	22.40±2.25^{a}	69.44±2.21^{a}	219.17±0.33^{a}	410.25±16.81^{ab}	0.15±0.02^{a}	1.92±0.10^{ab}
T_3	9.12±0.0 ^{a}	27.27±4.34^{ab}	79.17±4.17^{ab}	218.03±2.06^{a}	511.87±31.98^{b}	0.26±0.03^{b}	1.52±0.09^{a}
T_4	9.12±0.10^{a}	24.51±1.34^{ab}	77.78±3.67^{ab}	219.73±1.90^{a}	455.15±2.75^{ab}	0.17±0.015^{ab}	1.74±0.02^{ab}
T_5	9.11±0.01^{a}	32.18±3.01^{b}	72.22±1.39^{ab}	217.25±1.45^{a}	455.27±15.57^{ab}	0.16±0.03^{a}	1.73±0.06^{ab}
T_6	9.08±0.01^{a}	21.63±0.56^{a}	69.44±5.00^{a}	218.00±0.40^{a}	396.36±2.49^{a}	0.14±0.006^{a}	1.97±0.01^{b}

C=Control, T_1 Rice Polish, T_2 =Wheat Flour, T_3= Sweet potato, T_4=Yam, T_5: Tapioca, T_6: Rice husk
(Values are mean ± standard error, those that are statistically significant are presented as a, b, ab)

Table 4: Plankton Abundance

Treatment	Phytoplankton			Zooplankton			Protozoa	
	Bacillariophyceae	Coscinodiscophyceae	Cynophyceae	Chlorophyceae	Brachionidae	Vorticellidae	Parameciidae	Euglenoidea, Ciliate
C	Nitzschia	Cosinodiscus	Anabaena	Chlamydomonas, Scenedesmus	Brachionus	Vorticella	Paramecium	Euglena, Ciliate
T_1	Nitzschia	Cosinodiscus	-	Scenedesmus	Brachionus	Vorticella	Paramecium	Ciliate
T_2	Nitzschia	Cosinodiscus	Anabaena	Scenedesmus	Brachionus	Vorticella	Paramecium	Euglena, Ciliate
T_3	Spirulina, Meloseria	Cosinodiscus	-	Pediastrum, Scenedesmus Chlorella,	Brachionus	Vorticella	Paramecium	Ciliate
T_4	Spirulina, Meloseria	Cosinodiscus	-	Pediastrum, Scenedesmus Chlorella	Brachionus	Vorticella	Paramecium	Ciliate
T_5	Navicula, Meloseria Cyclotella	Cosinodiscus	-	Pediastrum, Scenedesmus Chlorella	Brachionus	Vorticella	Paramecium	Ciliate
T_6	Nitzschia	Cosinodiscus	-	Scenedesmus	Brachionus	Vorticella	Paramecium	Ciliate

C=Control, T_1 Rice Polish, T_2 =Wheat Flour, T_3= Sweet potato, T_4=Yam, T_5: Tapioca, T_6: Rice husk

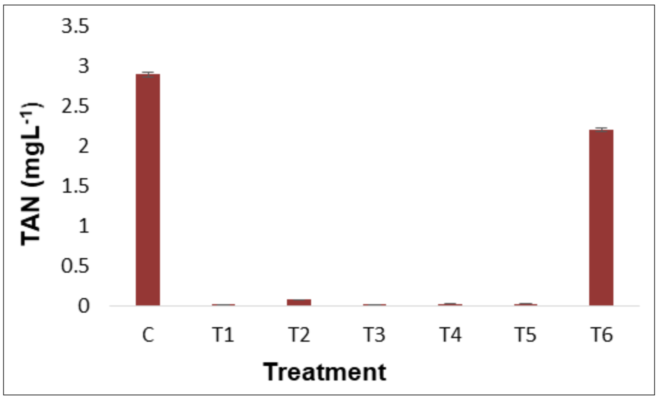

Fig 1: TAN concentration between the treatments

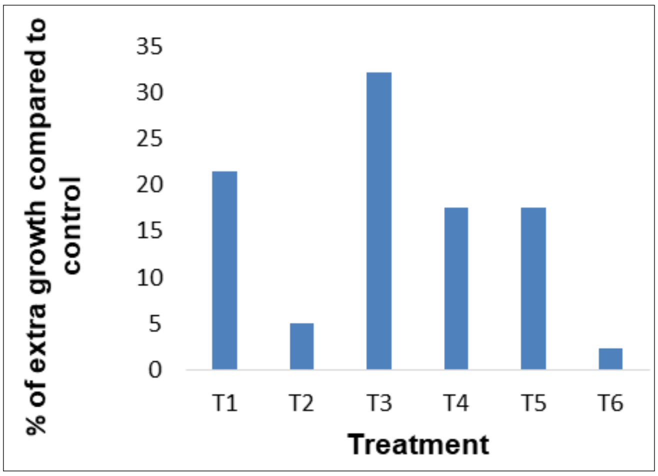

Fig 2: Percentage of extra growth in biofloc treatment system

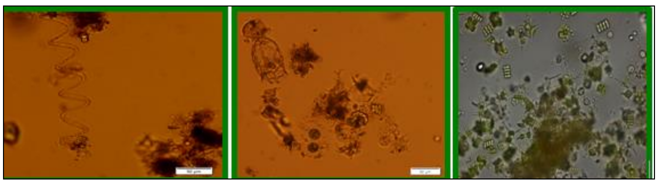

Fig 3: Association of biofloc, phytoplankton and zooplankton

Conclusion

The present study indicates that the addition of carbohydrate source with low protein diet at specific ratio could improves the water quality and enhance the growth of common carp. The tuber crop based biofloc system showed better growth and plankton abundance. The tuber crops can be easily grown at slope of the hills in the northeastern states of India and may use as a carbohydrate supplementation in fish culture to promote fish growth and increase the productivity of culture system with low protein diet. It can be concluding that biofloc technology may reduce the feed cost by lowering the protein demand and improves the water quality in high stocking density culture system.

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